

February 22, 1950.

Dear Max:

Your 2/18 series arrived in good shape about noon yesterday. Unfortunately, series C, F and H were started from segregant cells. Too bad about H especially, which looks to have been a long session.

Of the others, no segregations were observed in A, B, or E. However, G3 is a segregant; 11, 12, 14 are diploid.

I am still collecting some detailed data on the segregation pattern of H-226. There is very little (but definitely some) crossing over, and the diverse progeny of the last series clone G12: are the typical segregants. Mal+ seems to be considerably more frequent than Mal-, among single Lac- segregants individually ~~selected~~ picked at random from single segregating colonies.

I just sent you another package of screw cap vials to keep you current on your supply. Do you need any more? Is there anything else I can send you? Would it help if I sent you back the vials already sterilized, and with the nutrient broth in them?

As for the problem of picking diploid cells, I have found that good concentrations of them in liquid medium can be gotten by growing them in the following: (after Bernie Davis) (For H-226)

(per liter:)	K_2HPO_4	7
	KH_2PO_4	2
	Sod. citrate	0.5
	$MgSO_4 \cdot 7H_2O$	0.1
	$(NH_4)_2SO_4$	1
	lactose	Ø 1.0 (preferably autoclaved separately).

The final pH after growth is still about 7, and dense grown suspensions will keep quite well for at least a week in the refrigerator, and probably for much longer. As much as 95% diploid cultures are the usual result of inoculating from a single EMS Lac colony.

Under separate cover, I am sending a vial with such a suspension, as well as some dry mix to facilitate making up the medium. Lactose will have to be added, from a sterile 10% solution, 1:100.

In a week or two, I hope to be able to send you a draft outline for my conception of a paper for J. Bact. This weekend, however, I'm going down to Oak Ridge to give a seminar. [Rumors that I've accepted a position down there are definitely previous! — but thanks for your friendly advice].

Sincerely,

Joshua Lederberg